

Post Harvest Storage and Processing Changes in Carotenoids and Micronutrients in Fluted Pumpkin (*Telferia occidentalis* Hook F.)

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Abstract: The aim of this study was to investigate the effects of storage and processing on the carotenoids, vitamins and minerals of *Telfairia occidentalis* (Fluted Pumpkin) leaves. High Performance Liquid Chromatography (HPLC) was used to analyze the contents of the carotenoids in raw, stored and processed vegetables. The results indicated that *T. occidentalis* was rich in lutein (655.70µg/gdwt) and total β-carotene (230.82µg/gdwt). Thermal processing significantly ($p > 0.05$) increased the contents of carotenoids. Beta-carotene isomerized during thermal processing. The storage conditions resulted in non-significant increase in the contents of the carotenoids. Preliminary nutritional data including the mineral elements (K, Ca, Mg, Zn and Fe) and vitamins content (Ascorbic acid, riboflavin, thiamin and niacin) indicated that *T. occidentalis* is a good source of iron (8-9 mg/100g edible portion), ascorbic acid (88 – 160 mg/100g edible portion) and vitamin K (115 – 125 mg/100g edible portion). Cooking decreased the contents of water soluble vitamins and minerals. The concentrations of lutein, β-carotene and certain micronutrients in *T. occidentalis* are much higher than typical contents in conventional edible leafy vegetables. The results of this study therefore provide evidence that fluted pumpkin leaf could be important contributor in reducing hidden hunger in populations that consume adequate amounts of this vegetable.

Keywords: micronutrients, pro-vitamin A, *Telferia occidentalis*, processing, storage,

I. Introduction

Telfairia occidentalis Hook f. (Fluted Pumpkin) belongs to the family *Cucurbitaceae*. It is a tropical vine grown in West Africa as a leaf vegetable and for the edible seeds. The tender shoots, succulent leaves and immature seeds are cooked and consumed as vegetable [1]. Fresh leafy vegetables contribute vitamins, minerals and biologically active compounds [2]. Leafy vegetables also contain photosynthetic pigments (chlorophylls and carotenoids) [3]. These pigments produce specific colors on the food, which is one of the assessed visual quality attributes [4]. However, the functional components of the pigments play important roles in human health [5]. For example carotenes are the sources of vitamin A. Lutein and zeaxanthin are important factors for human vision [6]. Carotenoids and chlorophylls play important roles in the prevention of various diseases associated with oxidative stress, such as cancer, cardiovascular diseases and other chronic diseases. Vegetables are one of the most cost-effective and sustainable solutions to micronutrient deficiencies, which affect far more people than hunger alone, and this is crucial in most of sub-Saharan Africa [7].

The aim of this study is to evaluate carotenoids vitamins and minerals in *T. occidentalis* given different processing and storage treatments. Also, vegetables are often cooked before consumption in Africa. As cited in several literatures, leafy vegetables have been used in various micronutrient (especially Vitamin A) intervention programmes in several countries [8]. The results of this study will therefore determine whether fluted pumpkin leaf can potentially contribute to the alleviation of micronutrient deficiency in vulnerable populations. This will help to adequately establish its importance in human nutrition and provide basis for improved utilization of the crop.

II. Materials and methods

2.1 Collection of Sample: The crop *Telfairia occidentalis* was planted (October 2011) and harvested from two farms (in Enugu State, Nigeria) in December 2011. Natural organic manure was used as fertilizer for the crop. It was selected at random from the plant area and picked by hand mid-morning during the harmattan season. A minimum of 1kg of the crop was collected randomly from different plants within the field. The leaves were placed in black polyethelene bags and transported to Biochemistry Department of the University of Nigeria Nsukka for processing and analysis. Analyses of carotenoids on the dried and milled sample were carried out at IITA (International Institute of Tropical Agriculture) Ibadan, Nigeria.

2.2 Experimental Design: The experiment design was a randomized complete block design having vegetable type (1) and processing treatments (3) as some of the variations (giving 1 x 3 observations). Each observation was repeated three times (giving 3 x 3 = 9 observations) for each parameter tested

2.3 Processing of Samples: The edible and inedible portions of the samples were separated. The inedible portions were discarded. The edible portions were washed with tap water and were subsequently divided in three equal sub-samples. The first sub-sample was cooked for 5mins in boiling water with the lid on. The second sub-sample was wrapped in a newspaper and stored in the dark for 5 days at room temperature (29±2⁰C), while the third sub-sample was analyzed raw. The vitamins analyzed were ascorbic acid, riboflavin, thiamin, niacin and vitamin K. The minerals analyzed were potassium, calcium, magnesium, zinc and iron

2.4 Carotenoid Analysis : Both the raw, cooked and stored samples were oven dried in glass trays at 50⁰C for about 48 h until there was no further moisture loss. The dried leaves were milled and sieved through a 1mm stainless steel sieve to obtain a homogenized sample. Approximately 30g of each of the sieved powdered sample was stored in a sealed polyethylene bag and coded. The samples were stored at -20⁰C until they were analyzed for carotenoids.

2.5 HPLC Determination of Carotenoids : The method of Howe and Tanumihardjo [(9) was used. A Waters HPLC system (Water Corporation, Milford, MA) consisting of a Guard-column, C₃₀ YMC carotenoid column (4.6 X 250mm, 3µl) water 626 binary HPLC pump, 717 auto sampler and a 2996 photodiode array detector was used for carotenoid quantification. Chromatograms were generated at 450nm. Identification of lutein, β-cryptoxanthin, and β-Carotene were carried out using standards and with verification of absorption spectrum. Standard curves for lutein, β-cryptoxanthin and β-carotene standards already established in the crop utilization laboratory of IITA Ibadan, Nigeria were used.

2.6 Spectrophotometric Determination of Total Carotene Content: Determination of total carotene content of the leaf samples was according to the method of Rodriguez-Amaya and Kimura [10]. The absorbance was read at 450nm using Jenway Spectrophotometer (Model 752, England).

Total carotene content. (µg/g)

$$= \frac{A_{fr1} \times \text{volume (ml)} \times 10^4 \times (DF)}{A_{1cm}^{1\%} \times \text{sample weight}} \times 1.25$$

Where,

A_{fr1} = Absorbance at 450nm

Volume (ml) = volume of fraction 1 (5ml)

$A_{1cm}^{1\%}$ = 2592 (absorption coefficient of β-carotene in petroleum ether (P.E))

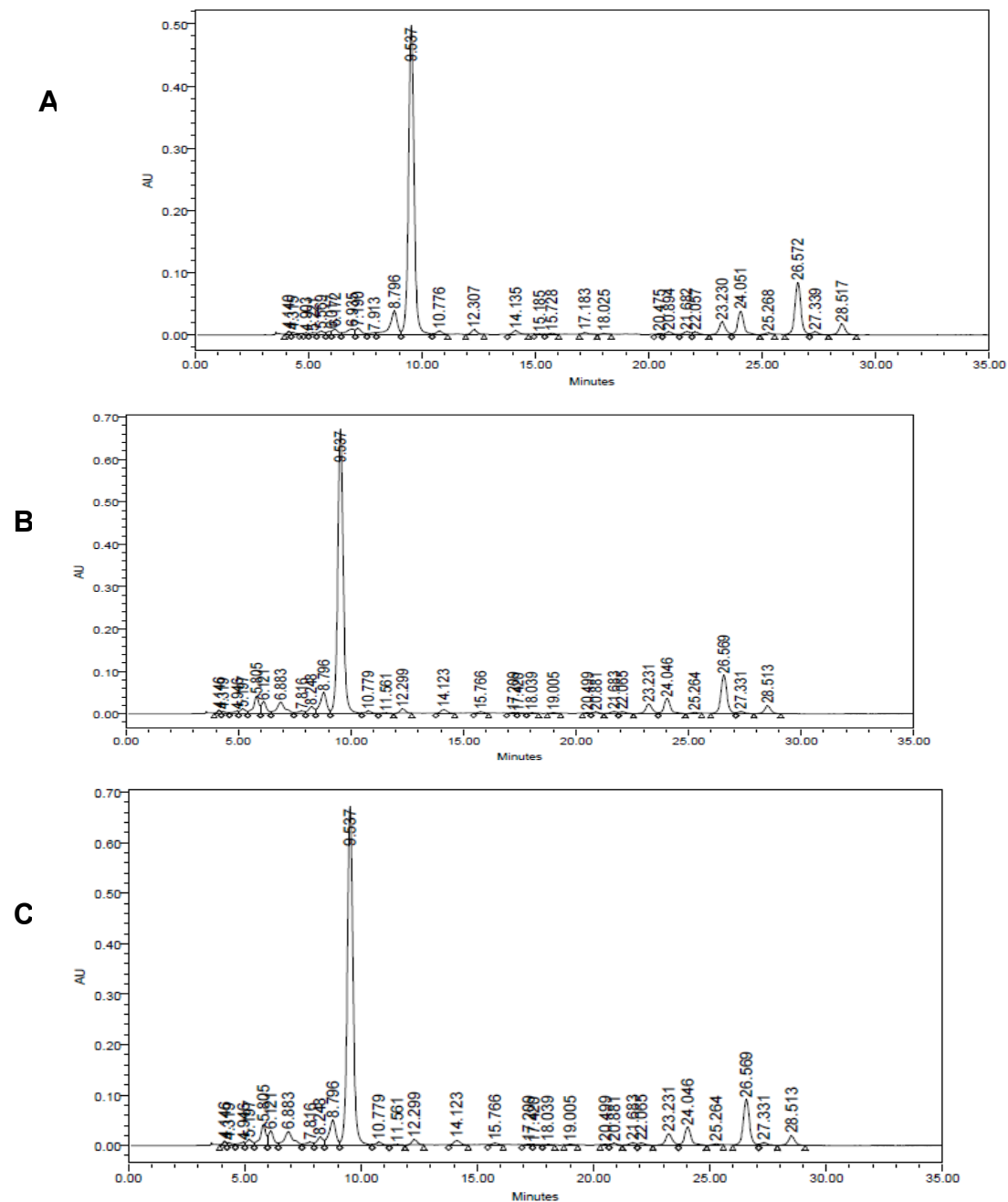
2.7 Determination of Vitamins: Vitamin content of *Telfairia occidentalis* leaf was determined by standard AOAC methods[[11].

2.8 Determination of Minerals: Mineral content was determined after wet digestion of samples using standard AOAC methods [11]. The solution obtained after wet digestion was evaluated for its content of potassium, calcium, magnesium, zinc and iron. Atomic Absorption Spectrophotometer (AAS) (Buck Scientific 210VGP) was used for the detection of the minerals.

2.9 Statistical Analysis: Analysis of data was performed using the Statistical Package for Social Science (SPSS) version 17. Analysis of Variance was specifically performed to detect significant differences ($P \leq 0.05$) among the sample means followed by the application of Least Significant Difference test (LSD) for the separation of significant means.

III. Results

3.1 Chromatographic Profiles of Carotenoids: The chromatogram of the carotenoids in *T. occidentalis* is presented in figures 1a-c. Two classes of carotenoids (xanthophylls and carotenes) were identified and quantified. Carotenoids were eluted in the following order: Lutein, β-cryptoxanthin, 13-*cis* β-carotene, 15-*Cis*-β-carotene, *trans*- β-carotene and 9-*cis*- β-carotene/ Peaks were more pronounced in the cooked sample (B)



(C) Stored
Figure 1: Carotenoid Profile of *Telfairia occidentalis* leaf samples by HPLC (A) Raw (B) Cooked

Total β-carotene content

Table 1 shows the total β-carotene concentrations of *T. occidentalis* leaf as a result of storage and cooking treatments. The total β-carotene (Tβ-c) level was significantly ($P < 0.05$) higher in cooked leaf ($532.66 \mu\text{g/gdwt}$) than in raw leaf ($230.82 \mu\text{g/gdwt}$). Table 1 also shows a non-statistical increase in stored leaf Tβ-C content ($245.42 \mu\text{g/gdwt}$) when compared with the raw leaf ($230.82 \mu\text{g/gdwt}$). This could result from continuation of physiological reactions [12].

Table 1 Effect of storage and processing methods on carotenoid content of *Telferia occidentalis* leaves

Carotenoids	Treatment		
	Raw	Cooked	Stored
Lutein	$655.70^b \pm 4.95$	$1158.83^a \pm 0.06$	$885.65^b \pm 2.36$
β-crypto xanthin	$5.17^b \pm 0.07$	$7.13^a \pm 0.16$	$4.99^b \pm 0.14$
9-cis-β-carotene	$26.47^b \pm 2.29$	$50.78^a \pm 1.05$	$28.50^b \pm 0.72$
13-cis-β-carotene	$32.03^b \pm 1.39$	$90.72^a \pm 0.10$	$35.97^b \pm 0.11$

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15-cis- β- carotene	54.65 ^b ± 0.62	121.67 ^a ±2.19	53.34 ^b ± 0.50
Trans- β- carotene	117.68 ^b ±2.18	269.48 ^a ±0.69	127.6 ^b 1± 2.23
Total- β- carotene	230.82 ^b ± 4.96	532.66 ^a ± 13.94	245.42 ^b ± 7.96 (LSD _{0.05} =2.03)

The β-cryptoxanthin (a xanthophyll) content of the leaf was relatively small (Table 1) β-cryptoxanthin is a minor provitamin A constituent of leaves [(13). The β-cryptoxanthin content of the cooked leaves (7.13µg/gdwt) was significantly (P>0.05) higher than in both the raw (5.17µg/gdwt) and stored (4.99µg/gdwt) leaf samples.

Lutein content of the cooked leaf (1158.83µg/gdwt) was significantly (p<0.05) higher than in both the raw (655.70µg/gdwt) and stored (885.65µg/gdwt) leaf samples (Table 1).

3.2 Provitamin A Content: The Provitamin A content of the leaf sample was calculated (Table 2). The provitamin A content of the raw, cooked and stored leaf sample were 233.41, 536.22 and 247.91µg respectively.

Table 2 Pro-vitamin A values of cooked and stored *Telferia occidentalis*

Provitamin A (µg/g)	% trans of total β-Carotene	% Retention (Total β-Carotene)
233.41	51.0	-
536.2	50.6	230.76 (after cooking)
247.91	52.0	106.32 (after storage)

Values are means ± standard deviations of duplicate determinations on dry weight basis. Means with different superscripts within the same column are significantly different (P≤ 0.05)

3.3 Mineral Content: The effects of storage and processing on selected minerals of *T. occidentalis* is presented in Table 3. The mineral contents of the raw leaves were K. 3.45; Ca. 1.50; Mg, 4.30; Zn, 2.00 and Fe 8.90 (mg/100g respectively).

Table 3: Effects of storage and processing methods on selected mineral content of *Telfairia occidentalis*

Mineral content (mg/100g edible portion fresh weight basis)					
Treatment	K	Ca	Mg	Zn	Fe
Raw	3.45 ^b ±0.07	1.50 ^b ±0.14	4.30 ^b ±0.14	2.00 ^b ±0.28	8.90 ^b ±0.14
Cooked	3.20 ^a ±0.00	1.00 ^a ±0.14	4.05 ^a ±0.21	1.70 ^a ±0.14	8.05 ^a ±0.35
Stored	3.35 ^b ±0.07	1.47 ^b ±0.07	4.23 ^b ±0.07	2.0 ^b ±0.07	8.74 ^b ±0.35

LSD=3.81

Values are mean±standard deviation of triplicate determination on fresh weight basis means with different superscripts within the same column are significantly different (p=0.05).

3.4 Vitamin Content: The vitamin content of *T. occidentalis* leaves is shown in Table 4. Vitamin concentrations (in mg/100g) were 160.15, 2.07, 0.08 and 2.38 for ascorbic acid, riboflavin, thiamin and niacin respectively, while vitamin K₁ content was 210µg/100g. Cooking significantly (P<0.05) decreased the contents of the water-soluble vitamin [13]. However, cooking increased significantly (P<0.05) the content of vitamin K₁ in the leaf.

Table 4: Effects of storage and processing methods on the content of selected vitamin of *Telfairia*

occidentalis leafy vegetable

Vitamin content per 100g edible portion, fresh weight basis					
Treatment	Ascorbic Acid (mg)	Riboflavin (mg)	Thiamin (mg)	Niacin (mg)	Vit. K (µg)
Raw	160.15 ^b ±0.07	2.07 ^b ±0.01	0.08 ^b ±0.01	2.38 ^b ±0.01	121 ^b .05
Cooked	88.10 ^a ±0.07	1.02 ^a ±0.01	0.04 ^a ±0.01	0.50 ^a ±0.00	125 ^a .67
Stored	99.27 ^a ±0.01	1.39 ^a ±0.07	0.04 ^a ±0.01	1.49 ^b ±0.00	115 ^a .98

LSD_{0.05}=4.05

Values are means ± standard deviations of duplicate determinations on fresh weight basis. Means with different superscripts within the same column are significantly different (P≤ 0.05)

IV. Discussion

The results of carotenoid analysis obtained from this study correlates with the results observed by Faber *et al.* (14). Processed samples had higher T β -c levels (Rodriguez-Amaya [15]. Cooking also resulted in 230.76% apparent T β -c retention when compared with the raw leaf. (Table1). Dietz and Erdman [16] reported that cooking resulted in greater than 100% retention of β -carotene in vegetables, because denaturation of carotene binding proteins releases the carotenoids so that they can be extracted more easily.

The percentage *trans*- β -carotene (Table 1) was lower in the cooked than in raw leaves. During cooking, some of the *trans*- β -carotene could have been converted to *cis*-isomers or other oxidation products [17]. The consequences of *trans-cis*-isomerization are changes in bioavailability and physiological activity [18,19]. The observed increase in T β -c during storage resulted in an apparent retention of 106.32% (Table 1) indicating that the storage conditions in our study did not degrade the carotenoids. Comparing the T β -c content of *T. occidentalis*, with previous reports, Žnidarčič *et al.* [20] recorded 70.1 μ g/gfw and 79.6 μ g/gfw in wild rocket and Garden rocket respectively. It seems therefore, that the Fluted pumpkin leaf is a very rich dietary source of β -carotene.

The most abundant *cis*-isomer of β -carotene in the raw, stored and cooked samples was 13-*cis*- β -carotene (Table 1). Out of the several different geometric isomers of β -carotene that exist in food and human tissues, the major β -carotene isomers in the circulation of humans are *trans*- β -carotene, with small amount of 13-*cis*- and 9-*cis*- β -carotene (21). Several researchers have observed high lutein content in green leafy vegetables [13, 22]. According to Wisniewka and Subczynski [6], the presence of lutein and/or zeaxanthin in the diet may be beneficial for reducing the incidence of the two common eye diseases of ageing, age related macular degeneration and cataracts formation. From our results, consumption of 50 – 70g/day of the cooked leaves analyzed in our study would meet the recommended daily allowance (RDA) of 900RE/day for men and 700RE/day for women, 19 – 30 years old (Souzan and ABD EL-AAI [23]. Higher values for calcium and potassium were reported by Agte *et al.* [13] in watercress Kale and cabbage and by Uusiku *et al.* [22] in *Amaranthus spp* and *Solanum nigrum* leaves respectively. Cooking for 5 min at 100°C significantly (P<0.05) reduced mineral content. These minerals leach into cooking water. The reductions could also result from effects of oxidizing agents, exposure to heat, light, and extremes of pH and other factors that affect organic nutrients [23]. The potassium, calcium and iron values in *T. occidentalis* correlated well with the findings of Sobowale *et al.* [24] Post-harvest storage conditions resulted in non-significant reductions in the mineral contents when compared with the raw leaves.

The results for mineral analysis of fluted pumpkin suggest the consumption of large quantities to meet the recommended daily allowance (RDAs) for minerals. However, Iron content can be considered adequate when compared with an RDA of 8mg Fe/day for men (19 – 30 years) and 18mg Fe/day for women (19 – 30 years) [25]. Vitamin K₁ is located in chloroplasts in plants, cooking by boiling may disrupt the cell wall, thereby releasing vitamin K₁ for measurements [13]. The loss of ascorbic acid observed in this study was 44%. The reported cases of ascorbic acid loss during blanching or cooking are enormous and may vary between 40 and 70% in some cooked vegetables when processed at 100°C for 10 min. [

] Also at the end of the storage period, the loss in ascorbic acid was 38%. Other researchers have also reported postharvest losses in ascorbic acid. 29-50% and 34-38% (24) for *Cassia tora* and *Corchorus tridens* leaves stored at 20°C for 8 days. Ascorbic acid, riboflavin and vitamin K in *T. occidentalis* are high and adequate to meet the RDAs of 90mg/day ascorbic acid, 1.3mg/day, riboflavin and 90 μ g/day vitamin K₁ in children and adults respectively.

V. Conclusion

The potentials of *T. occidentalis* in alleviating micronutrient deficiencies were evaluated in this research work. Results showed that the consumption of about 50g per day of cooked fluted pumpkin leaf would meet the required daily allowance (RDA) for Vitamin A in children and adults. Consumption of about 100g of pumpkin leaf per day would meet the RDAs of riboflavin, ascorbic acid and vitamin K in children and adults respectively. Storage and cooking of the leaves after harvest resulted in changes in carotenoids, vitamins and minerals in the leaves. The levels of nutrient retention after domestic processing support the inclusion of this leaf in a daily diet to overcome vitamin A deficiency, iron deficiency anaemia and age-related macular degeneration.

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